Original Article

Evaluation of antitussive and anti-asthmatic activity of *Tabernaemontana divaricata* (L.) R. Br. Ex Roem. and Schult

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Abstract

Background: The study was aimed to investigate the antitussive and anti-asthmatic activities of ethanolic extract of *Tabernaemontana divaricata* (TDEE) leaves by *in vivo* and *in vitro* models. Recently, indole alkaloids (monoterpenoid indole alkaloids) have been approved as investigational new drug for clinical trial in respiratory diseases, and *T. divaricata* has already proven its potential for the presence of indole alkaloids. **Materials and Methods:** Acute toxicity studies of TDEE were performed in accordance with the Organization for Economic Cooperation and Development guidelines no. 425. The sensitized guinea pigs were screened out and divided into control, standard, and TDEE-treated groups. Anti-asthmatic activity of TDEE was assessed by *in vitro* guinea pig tracheal chain method and *in vivo* bronchoprotective test method using aminophylline as a standard drug. Taken codeine as standard, antitussive activity was evaluated by *in vivo* citric acid-induced tussive response. **Results:** TDEE was found to be safe up to 2000 mg/kg, body weight. TDEE exhibits maximum bronchi relaxation of 91.66% and 92.83% against acetylcholine and histamine-induced contraction, respectively. TDEE exhibited maximum and significant ($P < 0.001$) bronchoprotection of 42.28% at the dose level of 200 mg/kg, body weight. TDEE at aerosolic dose of 6% (w/v) exhibited decreased average cough frequency (4.83 ± 0.30) which is quite significant ($P < 0.001$) and effective as compared to standard drug codeine. Based on the histopathological evidences, TDEE-treated groups showed reduced inflammatory cell infiltration and had restored epithelial damage. **Conclusion:** The results of the study revealed the potent antitussive and anti-asthmatic activities of *T. divaricata,* which support its further implication for the treatment of cough-associated complications such as cough variant asthma.

Keywords: Acetylcholine, aminophylline, bronchoprotection, codeine, histamine, *Tabernaemontana divaricata*

Introduction

Asthma is a chronic inflammatory disease characterized by the association of bronchial hyperresponsiveness, inflammation, and remodeling.[1-3] Current medications are effective in treating acute airway narrowing and decreasing inflammation but are relatively less effective in preventing chronic structural changes.[4] Asthma affects as many as 334 million people in the world.[5] Further, avoidable asthma deaths are still occurring due to inappropriate management. The etiological basis for effective management of asthma needs bronchodilator action as muscle relaxant medication, anti-inflammatory as preventive medication, and long-acting β2-agonist for symptomatic control.[5,6]

Alkaloids are the leading pharmacological active phytoconstituents which have a nitrogen atom in a heterocyclic ring structure. About 12,000 different types of alkaloids are produced by the diverse species of plants, and a quarter from this large group of natural product accounts for indole alkaloids. Complex structures and diverse pharmacological activities have made the research on indole alkaloids more interesting and attractive including the investigation of biosynthetic pathways for many decades.[7]

The ethnopharmacological relevance of *Tabernaemontana divaricata* reveals its antioxidant, anti-bacterial, anti-carcinogenic, analgesic and the enhancement of cholinergic activities in both peripheral and central nervous

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systems.[8] *T. divaricata* also possess immunomodulatory effect as it decreases the cytokine expression of interleukin-1 beta and tumor necrosis factor alpha-α (TNF-α) in human, *in vivo* and *in vitro* murine models, respectively.[9,10] This supports a novel approach for the treatment of allergic asthma via inhibition of the T-helper 2 derived cytokine expression, resulting in downstream effects on immunoglobulin E and eosinophils.[11] Studies showed that expression of TNF-α is increased at both protein and mRNA levels in different inflammatory cells such as T-cells, eosinophils, mast cells, and macrophages in asthmatic airways.[12] It has been also demonstrated that TNF-α is also responsible for the release of inflammatory mediators from human lung mast cells via the activation of transcription factors, such as nuclear factor-κB and activator protein 1.[12,13] Increased expression of various inflammatory system genes leads to increased synthesis of inflammatory cytokines such as interleukin 10, interleukin 12, interferon γ, inflammatory receptors and adhesion molecules.[14] The cough is considered to be the most common complication associated with the respiratory diseases such as asthma, bronchitis, and pneumonia, and the treatments available bring some inevitable side effects that synergize complications.[15] A number of alkaloids from the different plant sources have been found to possess antitussive activity.[16-18]

As a diverse source of alkaloids, the main objective of the study was to elucidate the pharmacological potential of *T. divaricata* for antitussive and anti-asthmatic activities so that it can be further implicated as the drug of choice for cough-associated respiratory complications such as cough variant asthma.

Materials and Methods

Drugs and chemicals

Gallic acid, quercetin, folin-ciocalteu reagent, and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma-Aldrich, St. Louis, USA. Acetylcholine chloride (CAS number 60-31-1), histamine dihydrochloride (CAS number 56-92-8), aminophylline (CAS no. 317-34-0), and citric acid (CAS number. 77-92-9) were procured from HiMedia Pvt. Ltd. Mumbai, India.

Plant material

Fresh leaves of *T. divaricata* for the present study were collected in the month of January from Bhopal, Madhya Pradesh, India. Plant material was identified and authenticated by the Head of Department, Department of Botany, Saifia Science College, Bhopal, and a specimen voucher (443/Bot/Saifia/13) was deposited at the Department of Pharmacognosy, Truba Institute of Pharmacy, Bhopal, for future reference.

Extraction

The leaves were shade dried for 2 weeks and then pulverized to coarse powder and passed through sieve no. 20. Coarsely dried powder was first defatted with petroleum ether (60°C–80°C) for 72 h to remove fatty materials and then extracted with ethanol (95%) using Soxhlet apparatus for 36 h; the extract was collected and concentrated in vacuum under reduced pressure and the dried crude extract was stored at 4°C for further study.

Preliminary phytochemical screening

Ethanolic extract of *T. divaricata* leaves (TDEE) was subjected to various phytochemical tests for the identification of the phytoconstituents (carbohydrates, tannins, alkaloids, glycosides, flavonoids, steroids, proteins, and amino acids) present in the extract using standard procedures.[19,20]

In vitro free radical scavenging activity using 1-diphenyl-2-picryl-hydrazyl method

The free radical scavenging activity (FRSA) of TDEE was measured by DPPH method. 0.1 mM solution of DPPH was prepared in methanol, and 1 ml of it was added to different concentrations of TDEE (50, 100, 200, 400, and 500 μg/ml) and the final volume of 3 ml was made with methanol. The mixture was shaken vigorously and incubated at room temperature for 30 min. Absorbance of the resulting mixture was measured at 517 nm against methanol as blank, using a ultraviolet-visible spectrophotometer (Systronics, 2203, Japan).[21] Each sample was evaluated in triplicate and results were represented as mean. The ascorbic acid was used as a standard antioxidant in this method. Percentage of DPPH FRSA was determined as follows:

$$\% \text{ (FRSA)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

Animals

The experiment was carried out on healthy guinea pigs (400–600 g). The animals were acclimatized to the standard laboratory conditions, fed with standard pellet diet and water *ad libitum* during the study. The study protocol was approved by the Institutional Animal Ethics Committee as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India (Approval number 1196/a/08/CPCSEA).

Acute oral toxicity study

The acute oral toxicity study was evaluated as per the Organization for Economic Cooperation and Development (OECD) guidelines no. 425, on guinea pigs (400–600 g) of either sex. Before the experiment, animals were fasted overnight with water *ad libitum*. Six animals were selected which received a dose of 2000 mg/kg, body weight. All six animals were given a single dose of 2000 mg/kg, body weight of TDEE by oral gavage. Animals were observed individually for any sign of toxicity, behavioral changes, and mortality after dosing, with special attention given during the first 4 h, and thereafter for 24 h, for a total period of 7 days.[22]

Anti-asthmatic activity

In vitro method

Effect of *Tabernaemontana divaricata* on isolated guinea pig tracheal chain preparation

The male guinea pig was sacrificed by the overdose of sodium pentobarbital (100 mg/kg, body weight, i.p.) and was confirmed

by cervical dislocation to isolate tracheal rings and at least 5–6 rings were tied together by their cartilage portion to form a tracheal chain and 12–14 such preparations were transferred to a dish containing Krebs's solution. The chain was mounted in a 20 ml organ bath containing Krebs–Henseleit (K–H) solution ([mM]: NaCl, 118.4; KCl, 4.7; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; $MgSO_4$, 1.2; glucose, 11.1; pH 7.4 ± 0.05), at 35°C–37°C under 0.5 g tension and allowed to equilibrate at least for 1 h before commencing the experiment. During the experiment, the K–H solution was replaced after every 10 min. After the equilibrium period, contraction was induced by adding acetylcholine or histamine. Thereafter, standard aminophylline (1 µg/ml) and the TDEE at 2 mg/ml and 4 mg/ml were added serially as 0.2, 0.4, 0.6, 0.8, and 1 ml in cumulative doses and observed for bronchodilation using a Biopac MP 45, data acquisition system, respiratory transducers, and expressed as percent bronchorelaxation.[23,24]

In vivo method

Effect of *Tabernaemontana divaricata* on histamine and acetylcholine-induced respiratory distress: Bronchoprotective test

The sensitivity and suitability of the guinea pig for the study were screened by challenging each animal by an equal volume mixture of 0.1% histamine and 2% acetylcholine chloride in a histamine chamber under pressure of 450 ± 50 mmHg for 15 s. The onset of respiratory distress in seconds (preconvulsive) was carefully measured for each animal during an aerosol challenge. Animals having a pre-convulsive time >120 s were considered insensitive and unsuitable and not included in the study. The selected 24 male guinea pigs were divided into control, standard, and two test groups (n = 6). The control group animals received 5 ml/kg of 0.1% carboxy methyl cellulose (CMC), standard group animals received aminophylline (10 mg/kg, body weight) suspended in 0.1% CMC, and test group animals received 100 and 200 mg/kg, body weight of TDEE suspended in 0.1% CMC. All the treatments were given by the oral gavage route. Animals of all groups except control were pretreated with a single dose of their respective treatment (standard or test drug), daily for 3 days prior to the bronchial challenge while the last dose of the extract or standard drug was administered 1 h before the bronchial challenge. The delitescence/subsidence of convulsion and tumble numbers for each animal were recorded within a 6-min interval of exposure. Bronchial challenge by aerosol provoked a bronchospastic reaction in all the sensitive animals within 3 min. The delay in the appearance of the bronchospastic reaction was considered as bronchoprotective effect and expressed as percentage protection relative to the control group.[25]

$$\text{Percentage Protection} = 1 - \frac{(T1)}{(T2)} \times 100$$

Where T1 is the preconvulsive breathing time (s) in the control group; T2 is the preconvulsive breathing time (s) in the treatment or standard group.

Histopathological study

On the last day of the bronchoprotective test, lungs of guinea pig from each group were isolated; embedded in paraffin and tissue sections were stained using hematoxylin and eosin dye for histopathological studies.[26]

Effect of *Tabernaemontana divaricata* on *in vivo* citric acid-induced cough response

Non anesthelized and unrestrained 24 male guinea pigs were selected for the study and were divided into four groups (n = 6). Each animal was individually placed in a transparent chamber of 30 cm × 20 cm × 20 cm and exposed to nebulized aqueous solution of 0.1 g/ml of citric acid (nebulizing rate: 0.7 ± 0.04 ml/min) continuously for 7 min. During the last 5 min of exposure, each animal was observed continuously and closely to determine the number of cough responses and there was a clear difference between cough and sneezing in sound and behavior of the animal as well. The above protocol was performed for every animal from each group for 10 min after exposing animals to the aerosol solutions of saline (for a baseline measurement), codeine solution (0.03 g/ml, standard), TDEE (3% w/v), and TDEE (6% w/v) for at least 7 min and was observed for cough response.[27,28]

Statistical analysis

All the experimental results were represented as mean ± standard error of mean. Data was analyzed using one-way analysis of variance followed by Tukey–Kramer multiple comparisons test. Statistical calculations were carried out using GraphPad InStat 3 software, San Diego, CA, USA. $P < 0.05$ was considered statistically significant in all the cases.

Results

Phytochemical screening

Preliminary phytochemical investigation of TDEE revealed the presence of alkaloids, glycosides, flavonoids, tannins, triterpenoids, polyphenols, carbohydrates, and proteins [Table 1].

In vitro antioxidant activity of *Tabernaemontana divaricata* by 1-diphenyl-2-picryl-hydrazyl method

Table 1: Phytochemical screening of ethanolic extract of *Tabernaemontana divaricata* leaves

Chemical test	Inference
Carbohydrates	+
Tannins	+
Alkaloids	+
Glycosides	+
Flavonoids	+
Steroids and sterols	+
Proteins and amino acids	+

Plus (+) Sign indicates presence and minus (-) sign indicates absence of phytochemical compound

DPPH is a well-known stable free radical used for the evaluation of FRSA. Reduction of DPPH by antioxidant compounds present in the plant extract produces a color change reaction from purple to pale yellow. The reduction of DPPH-free radicals could be assessed by determining its absorbance at 517 nm using a spectrophotometer.[21] Although TDEE shows less antioxidant activity as compared to ascorbic acid, it was found to show a gradual increase in percentage inhibition with increasing concentration at 517 nm [Table 2] as an antioxidant with maximum inhibition of 64.85% at 500 µg/ml as compared to ascorbic acid (82.78%) at 500 µg/ml. TDEE exhibits significant difference ($P < 0.0001$) in antioxidant activity with corresponding concentrations in a dose-dependent manner as compared with ascorbic acid. Findings of *in vitro* FRSA study revealed the role of TDEE as a primary antioxidant and free radical inhibitor.

Table 2: Antioxidant activity of ethanolic extract of *Tabernaemontana divaricata* leaves by 1,1-diphenyl-2-picrylhydrazyl assay

Sample	Concentration (µg/ml)	Absorbance at 517 nm (mean±SEM)	Percentage inhibition
Ascorbic acid (standard)	50	0.473±0.004	14.31
	100	0.369±0.004	33.15
	200	0.237±0.003	57.06
	400	0.186±0.003	66.30
	500	0.095±0.002	82.78
TDEE	50	0.526±0.005	4.71
	100	0.420±0.003	23.91
	200	0.336±0.003	39.13
	400	0.236±0.002	57.24
	500	0.194±0.002	64.85

$P < 0.0001$ represents significant difference in percentage inhibition between ascorbic acid and TDEE with respect to their corresponding concentration. Data was analyzed by two-way ANOVA using Sidak's multiple comparisons test, all values were represented as mean±SEM (n=3) (control OD at 517 nm was 0.552). TDEE: Ethanolic extract of *Tabernaemontana divaricata* leaves, SEM: Standard error of mean, ANOVA: Analysis of variance, OD: optical densitiy

Acute oral toxicity study

Acute toxicity studies of TDEE were performed in accordance with the OECD 425, and the extract was found to exhibit a great margin of safety up to dose of 2000 mg/kg, body weight; there was no change in the behavioral pattern and no sign of toxicity and mortality was observed during the overall toxicity studies. Accordingly, 1/10th of this dose was considered to be the experimental safe dose.

Effect of *Tabernaemontana divaricata* on isolated guinea pig tracheal chain preparation

TDEE when added cumulatively to the organ bath containing either acetylcholine or histamine precontracted tracheal chain exhibited varying degrees of bronchorelaxation. TDEE exhibited maximum bronchorelaxation of 91.66% ($P < 0.0001$) and 92.83% ($P < 0.1$) against acetylcholine- induced and histamine-induced bronchocontraction, respectively, when compared with standard [Tables 3 and 4].

Effect of *Tabernaemontana divaricata* on histamine- induced and acetylcholine-induced respiratory distress: Bronchoprotective test

For the bronchoprotective test, first the sensitive guinea pigs were screened for the study by challenging them through an equal volume aerosolic mixture of 0.1% histamine and 2% acetylecholine chloride in a histamine chamber for 15 s. For this screening, 36 guinea pigs were randomly selected for aerosolic challenge and 33 animals were found sensitive and

Table 3: Acetylcholine induced contraction versus treatment

Group	Dose	Contraction versus treatment (ml)				
		0.2	0.4	0.6	0.8	1.0
Standard	1 µg/ml	38.16±0.54	56.83±0.65	93.5±0.56	97.16±0.47	98.09±0.30
TDEE	2 mg/ml	14.5±0.42****	26.16±0.65****	54.83±0.94****	70.33±1.20****	84.83±0.83****
	4 mg/ml	18.16±0.70****	38.33±0.55****	62.16±0.60****	91.5±0.42***	91.66±0.66****

****P<0.0001 and ***P<0.001 represents significant difference as compared with standard group, (at 0.8 ml of 1 µg/ml of standard, 38 mm was considered 100%). For bronchorelaxation study against acetylcholine, data was analyzed by one-way ANOVA using Tukey-Kramer multiple comparisons test, all values were represent as mean±SEM (n=4). TDEE: Ethanolic extract of *Tabernaemontana divaricata* leaves, SEM: Standard error of mean, ANOVA: Analysis of variance

Table 4: Histamine induced contraction versus treatment

Group	Dose	Contraction versus treatment (ml)				
		0.2	0.4	0.6	0.8	1.0
Standard	1 µg/ml	44.16±0.47	78.66±0.42	94.5±0.42	95.83±1.13	96.83±1.13
TDEE	2 mg/ml	15.33±0.71****	36.5±0.61***	60.66±0.61****	85.16±1.19****	86.66±1.28****
	4 mg/ml	21.66±0.61****	45±0.89***	82.83±0.90****	92±0.68*	92.83±0.70*

****P<0.0001, ***P<0.001 and *P<0.1 represents significant difference as compared with standard group (at 0.8 ml of 1 µg/ml of standard, 22 mm was considered 100%). For bronchorelaxation study against histamine, data was analyzed by one way ANOVA using Tukey-Kramer multiple comparisons test, all values were represent as mean±SEM (n=4). TDEE: Ethanolic extract of *Tabernaemontana divaricata* leaves, SEM: Standard error of mean, ANOVA: Analysis of variance

suitable for the study and the remaining three animals were found unsuitable for the study. After sensitivity screening, all the animals were re-randomized before treatment to reduce the error in-between the groups. In bronchoprotective test model, TDEE at the dose level of 100 and 200 mg/kg, body weight, showed significant bronchoprotection ($P < 0.0001$) with increased latency when compared with before treatment values [Figure 1a]. TDEE was also found to exhibit a potential bronchoprotection with reduced tumble numbers ($P < 0.001$) compared with control group [Figure 1b]. The percentage bronchoprotection exhibited by TDEE at 200 mg/kg, body weight, was quite significant as compared to the standard drug aminophylline (42.73%) so that we had obtained the order of potency, i.e., aminophylline ≥ TDEE at 200 mg/kg, body weight > TDEE at 100 mg/kg, body weight [Figure 1c].

Effect of test drug on *in vivo* citric acid-induced cough response

TDEE at 3% and 6% w/v aerosolic dose against citric acid-induced tussive reaction was found to exhibit a significant ($P < 0.001$) reduction in cough response as compared to control [Figure 2].

Histological study

Histological examination reveals that the lungs of the control (bronchial challenged with no treatment) group animals showed a marked population of inflammatory cells surrounded by the damaged epithelium in alveolar walls, perivascular and peribronchial spaces [Figure 3a]. TDEE treatment markedly reduced the inflammatory cell infiltration around the damaged epithelium [Figure 3c and d] as compared to control [Figure 3a] and standard [Figure 3b].

Discussion

Various cellular elements such as T-lymphocytes, mast cells, neutrophils, and epithelial cells play a pivotal role in the pathophysiology of asthma.[29] Due to the vast variety of the associated side effects of the currently available treatment options (bronchodilators and anti-inflammatory agents), herbal medicines proved their potential as safe and efficacious alternatives,[30] for the treatment of asthma which is also supported by some of the clinical studies.[31]

The present study was aimed to investigate the antitussive and anti-asthmatic activities of TDEE. Preliminary phytochemical screening of TDEE showed the presence of alkaloids, glycosides, flavonoids, tannins, triterpenoids, polyphenols, carbohydrates, and proteins while acute oral toxicity studies revealed the non-toxic nature of the extract for subsequent studies. We have also investigated the *in vitro* FRSA of TDEE by DPPH method and found that TDEE could act as a key antioxidant and free radical inhibitor which could be associated with the polyphenolics present in the extract.

Histamine is the most dominant factor for asthma[32] and an important mediator of bronchial muscle contraction and the obstruction of these may occur via H_1 receptors. In addition, acetylcholine released from efferent nerve endings of the inner bronchus results in the excessive formation

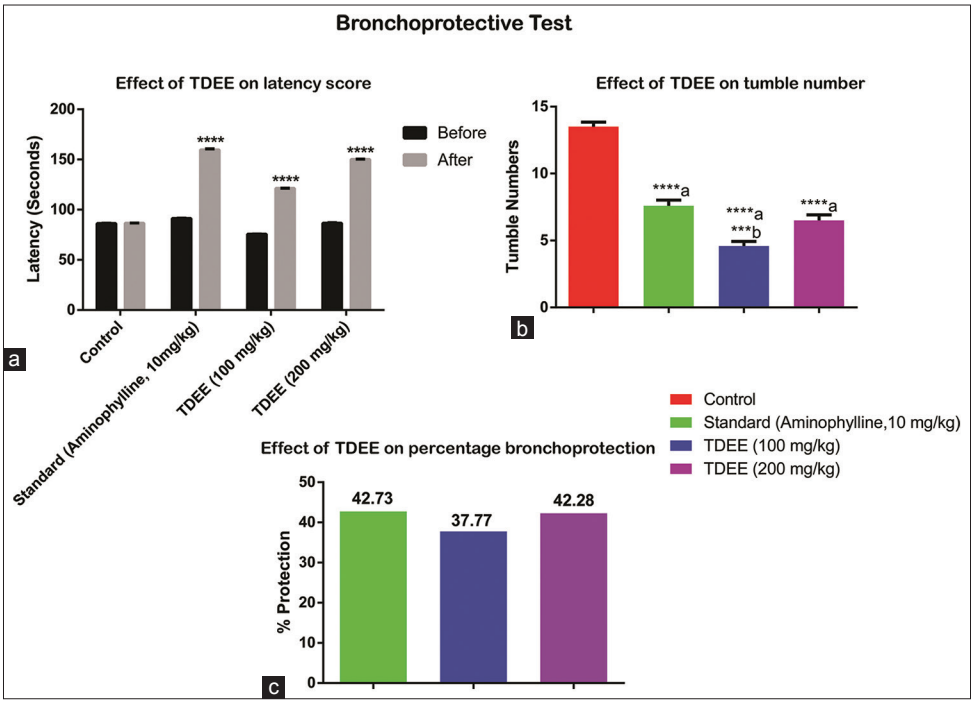

Figure 1: Bronchoprotective effect of *Tabernaemontana divaricata* against histamine and acetylcholine induced bronchospasm. (a) Results are represented as mean ± standard error of mean, $n = 6$ animals in each group, ****$P < 0.001$. (b) Results are represented as mean ± standard error of mean, $n = 6$ animals in each group, a- significant difference versus control group and b- significant difference versus standard group, ***$P < 0.001$, ****$P < 0.001$, (c) *Tabernaemontana divaricata* at 200 mg/kg, possess significant percentage of bronchoprotection as compared to aminophylline

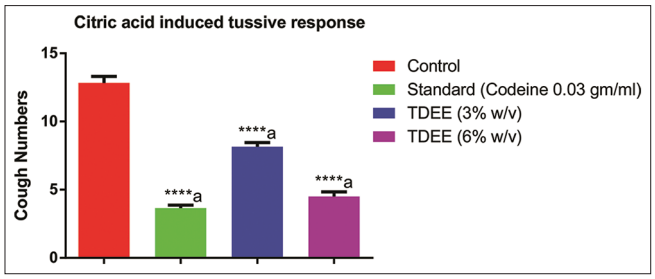

Figure 2: Antitussive effect of *Tabernaemontana divaricata* against citric acid-induced tussive response, all values are represented as mean ± standard error of mean, $n = 6$ animals in each group, data were analyzed by one-way analysis of variance using Turkey-Kramer multiple comparisons test, a- significant difference vs. control group and b- significant difference versus standard group, $***P < 0.001$, $****P < 0.001$

of inositol 1,4,5-triphosphate (IP-3) in bronchial muscles which leads to the intracellular release of calcium and initiate bronchoconstriction. It has been reported that bronchial acetylcholine and histamine H_1 receptor blockade results in bronchodilation, which is considered as vital in the treatment of asthma.[33] A prominent effect caused by both leads to a varying degree of bronchoconstriction that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms.[34]

The results of histamine induced and acetylcholine-induced tracheal chain contraction as well as bronchospasm depict the significant bronchorelaxant and bronchoprotective effects of TDEE, which suggest that the extract might have some histamine as well as cholinergic receptor antagonistic properties. Histological evidences also revealed the anti-inflammatory efficacy of the TDEE, namely, reduction in the inflammatory cell infiltration and repaired epithelium damage.

Cough is a very common complication that is mostly related to a number of pulmonary diseases. It is a normal physiological response to an irritation of the laryngo-tracheo-bronchial system caused by mechanical or chemical stimulation. It may be painful and fatiguing and require suppression by antitussive drugs. In animals, coughing has been elicited by various methods and mechanisms such as mechanically induced cough reflex in unanesthetized dogs, chemically induced cough response (sulfur dioxide-induced cough response) in guinea pigs and mice, ammonia-induced cough response in mice, and citric acid-induced coughing in guinea pigs.[35-39]

In the present study, the antitussive activity of TDEE has been compared with that of standard drug codeine against coughing induced by chemical stimulation (citric acid) in guinea pigs. Guinea pigs were selected for *in vivo* citric acid-induced cough due to their afferent nerve reaction and similarity in cough production in response to citric acid like in humans.[40] Tussive response induced by the citric acid was a result of the stimulation of the histaminergic and acetylcholinergic receptors followed by the bronchial hyperresponsiveness and hypersensitivity, tracheobronchial contraction and cough induction.[41] The extract was found to show significant inhibition of cough as compared with standard

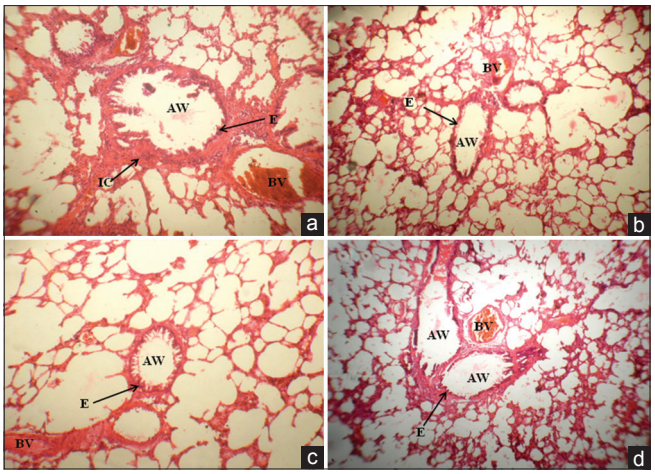

Figure 3: Histopathological sections of guinea pig lungs showing the effect of *Tabernaemontana divaricata*, (a) control group; epithelium (E); surrounded by peribronchial and perivascular inflammatory cells and blood vessels, (b) standard group; reduced epithelial damage with minimal inflammatory cells, (c) bronchial challenged group treated with *Tabernaemontana divaricata* (100 mg/kg); reduced inflammatory cells; improved airway with no epithelial repair, (d) Bronchial challenged plus treatment with *Tabernaemontana divaricata* (200 mg/kg) significant reduction in inflammatory cells, minute damaged epithelium was observed as compared to untreated group

treatment (codeine) in a dose-dependent manner. Alkaloids are widely reported for their mast cell stabilizing and α and β-adrenergic receptor stimulating property.[42,43] Phytochemical analysis of the TDEE confirmed the presence of alkaloid and polyphenolics. The antitussive mechanism of TDEE in citric acid induced cough response was probably due to the mast cell stabilizing property via the inhibition of histaminergic and acetylcholinergic receptors located in the bronchial tract or by the activation of α and β-adrenergic receptors.[44]

Excluding alkaloids, other important pharmacologically active secondary metabolites like polyphenolics, flavonoids and tannins are also thought to be key modulators of the histamine and acetylcholine-induced respiratory distress and tussive response by their well-known antioxidant, anti-inflammatory, expectorant and antimicrobial properties.[45-47] On the basis of findings of the present study, observed anti-asthmatic and antitussive activity may be related to the presence of alkaloids and other secondary metabolites present in TDEE.[38]

Conclusion

The results take the lead to provide the preliminary and pre-clinical experimental evidences demonstrating that the TDEE has anti-asthmatic activities in both *in vivo* and *in vitro* animal models and anti tussive activity in *in vivo* animal model. Even though the results obtained are not sufficient to prove the mechanism of the TDEE in the inhibition of allergic, inflammatory and tussive response. Further investigations are needed to clarify the exact bioactive phytoconstituents and the precise mechanism of the TDEE.

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Conflicts of interest

There are no conflicts of interest.

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हिंदी सारांश

चांदनी (टेबर्नीमोंटेना डाइवरीकेटा) के कासरोधक और दमा विरोधी प्रभाव का परीक्षण

अमित कुमार श्रीवास्तव, हेमंत नागर, रजनीश श्रीवास्तव, वर्षा अहिरवार, हरिनारायण सिंह चंदेल

प्रस्तुत अध्ययन चांदनी (टेबर्नीमोंटेना डाइवरीकेटा) के पत्तों के मद्यजन्य अर्क के कासरोधक और दमा विरोधी प्रभाव का परीक्षण करने के उद्देश्य से किया गया था। हाल ही में इण्डोल उपक्षार (अल्कलॉइड) को नई औषध अन्वेषण के रूप में सांस की बीमारियों में चिकित्सीय परीक्षण के लिए मंजूरी दी है और चांदनी ने इण्डोल उपक्षार की उपस्थिति के लिए अपनी क्षमता पहले से ही सिद्ध की है। चांदनी के पत्तों के मद्यजन्य अर्क (टीडीइइ) की तीव्र विषाक्तता का अध्ययन ओईसीडी ४२५ के अनुसार किया गया। संवेदनशील गिनी सूअरों को नियंत्रण, मानक और टीडीइइ इलाज समूहों में विभाजित किया गया। दमा विरोधी प्रभाव का परीक्षण गिनी सूअरों की सांस की नली श्रृंखला विधि द्वारा और श्वसनी सुरक्षात्मक परीक्षण विधि द्वारा किया गया, इन दोनों विधियो में अमीनोफाईलीन को मानक के रूप में लिया गया। कासरोधक प्रभाव का परीक्षण साइट्रिक अम्ल प्रेरित कासरोधक प्रतिक्रिया द्वारा किया गया, इस विधि में में कोडीन मानक के रूप में लिया गया। तीव्र विषाक्तता अध्ययन में टीडीइइ २००० मिली ग्राम/ किलो, शरीर भार तक सुरक्षित पाया गया। परीक्षण में टीडीइइ ने ऐसीटीलकोलीन और हिस्टामिन के विरुद्ध क्रमशः ९१.६६% और ९२.८३% की अधिकतम श्वसनी शिथिलन गतिविधि प्रदर्शित की। टीडीइइ ने अधिकतम और महत्वपूर्ण श्वसनरक्षण (४२.२८%), २०० मिली ग्राम/ किलो, शरीर भार मात्रा स्तर पर किया। टीडीइइ ऐरोसोलिक की ६% की मात्रा पर औसत खांसी की बारंबारता (४.८३±०.३०), में भी कमी आई जो की मानक दवा कोडीन की तुलना में प्रभावी है। टीडीइइ इलाज समूहों में उत्तेजक कोशिका रिसाव कम हुआ और उपकला क्षतिग्रस्तता भी कम हुई। अध्ययन के परिणाम बताते हैं कि चांदनी के पत्तों में कासरोधक और दमा विरोधी प्रभाव के लिए प्रबल गुण हैं और इनका खांसी संस्करण दमा (कफ वेरिएटअस्थमा)और खाँसी संबंधित जटिलताओं के उपचार के लिए भी प्रयोग किया जा सकता है।